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16S rRNA gene analysis

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An abbreviated version of this protocol was published in Science Immunology in Nov 2020

Gut microbiota–specific IgA+ B cells traffic to the CNS in active multiple sclerosis

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Detailed protocol

16S rRNA gene analysis

DNA was prepared from fecal samples using the PowerFecal DNA Isolation Kit (Qiagen). The V4 region of the 16S rRNA gene was amplified in triplicate as previously described (57). Triplicate reac-tions were combined and purified using the SequalPrep Normalization Plate Kit (Invitrogen) according to the manufacturer's specifications. Purified amplicons were quantitated using the Qubit dsDNA HS Assay
Kit and pooled at equimolar concentrations. The amplicon library was concentrated using the Agencourt AMPure XP System (Beckman Coulter) quantitated using the KAPA Library Quantification Kit (KAPA Biosystems) and diluted to 2 nM. Equimolar PhiX was added at 40% final volume to the amplicon library and sequenced in a paired-end 153 base pair (bp) × 153 bp format on an Illumina NextSeq500 machine. In total, 142 samples were sequenced (tables S1 and S2).
Raw sequence data were converted from bcl to fastq format using bcl2fastq v2.16.0.10. R1/R2 fastq data were merged using the program FLASH (Fast Length Adjustment of SHort reads) (58) with a minimum overlap of 25 bp. Merged pairs were then demultiplexed using "split_libraries_fastq.py" in QIIME (59) and passed through USEARCH (60) fastq_filter to remove any sequences that contain more than two expected errors.
Amplicon reads were assigned to OTUs using a closed-reference OTU picking protocol against Greengenes database (v13.8) at 97% identity by using QIIME (59). Taxonomy was assigned to each read by accepting the Greengenes taxonomy string of the best matching Greengenes sequence. The sequences were rarified to 70,000 reads per sample, and 134 samples were kept for downstream analyses. OTUs were filtered to retain the ones present in at least 5% of sam-ples, covering at least 100 total reads. a-Diversity and b-diversity were measured by Shannon index and weighted UniFrac distance, respectively. Paired and nonpaired differential analyses between IgA+ and IgA−, MS (relapse and remission patient subsets), and healthy controls were performed using DESeq2 R package (61) with Benjamini- Hochberg correction for multiple comparisons. OTU representative sequences were picked and aligned against Greengenes. The aligned sequences were then used to build the phylogenetic tree by the fasttree
method in QIIME. An ICI for each differential OTU (adjusted P value of <0.05) was calculated as follows: ICI = relative abundance (IgA+)/relative abundance (IgA−). PICRUSt1.0.0 (Phylogenetic In-vestigation of Communities by Reconstruction of Unobserved States) (62) was applied to predict metagenome content of the microbiota samples from the 16S rRNA profiles, and Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologies (KOs) were categorized. Differ-ential analysis of KOs between patients with MS and healthy controls was performed using the DESeq2 R package with Benjamini-Hochberg correction for multiple comparisons.
To identify enriched OTUs bound by each mAb, we divided the relative abundance of each OTU within the sample by the relative abundance within the whole microbiota bound by all antibodies. OTUs were kept only if the relative abundance was larger than 0.05% and enrichment score larger than 0.5 in any of the five mAb-bound sam-ples. The phylogenetic tree of enriched OTUs was built on the basis of the phylogenetic distance of the representative sequences of all

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1. Zhou, X. (2020). 16S rRNA gene analysis. Bio-protocol Preprint. bio-protocol.org/prep692.
2. Pröbstel, A., Zhou, X., Baumann, R., Wischnewski, S., Kutza, M., Rojas, O. L., Sellrie, K., Bischof, A., Kim, K., Ramesh, A., Dandekar, R., Greenfield, A. L., Schubert, R. D., Bisanz, J. E., Vistnes, S., Khaleghi, K., Landefeld, J., Kirkish, G., Liesche-Starnecker, F., Ramaglia, V., Singh, S., Tran, E. B., Barba, P., Zorn, K., Oechtering, J., Forsberg, K., Shiow, L. R., Henry, R. G., Graves, J., Cree, B. A. C., Hauser, S. L., Kuhle, J., Gelfand, J. M., Andersen, P. M., Schlegel, J., Turnbaugh, P. J., Seeberger, P. H., Gommerman, J. L., Wilson, M. R., Schirmer, L. and Baranzini, S. E.(2020). Gut microbiota–specific IgA+ B cells traffic to the CNS in active multiple sclerosis . Science Immunology 5(53). DOI: 10.1126/sciimmunol.abc7191

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